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Serum zinc concentrations and incident hypertension: new findings from a population-based cohort study

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Abstract

Objective: Zinc is an essential trace element that plays a key role in several cellular processes and has been suggested to be involved in blood pressure regulation. We aimed to prospectively investigate the association between baseline concentrations of serum zinc and incident hypertension.

Methods: We analyzed data involving 1,652 men aged 42-61 years without a known history of hypertension at baseline in the Kuopio Ischemic Heart Disease population-based cohort study, with the assessment of serum zinc concentrations. Hazard ratios (95% confidence intervals [CIs]) for incident hypertension were assessed.

Results: During a median follow-up of 24.7 years, 259 participants developed hypertension. Serum zinc was weakly correlated with several risk markers for hypertension and non-linearly associated with incident hypertension. In analyses adjusted for age, the hazard ratio (95% CIs) for hypertension in a comparison of the top quartile versus bottom quartiles 1-3 of zinc concentration was 1.65 (1.27 to 2.15; $P<0.001$), which was minimally attenuated on adjustment for several established risk factors 1.48 (1.13 to 1.93; $P=0.004$). The association remained unchanged on further adjustment for renal function, socioeconomic status, and dietary factors. The findings were generally consistent across several clinical subgroups. There was no evidence of an association of dietary zinc intake with risk of hypertension.

Conclusion: This prospective study suggests higher serum zinc concentration is positively and independently associated with incident hypertension in men. Further evidence from large-scale population-based studies is needed to support these findings and assess the mechanisms underlying the association.

Keywords: zinc; risk factor; blood pressure; hypertension; cohort

Abbreviations: BMI, body mass index; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; KIHD, Kuopio Ischemic Heart Disease; SBP, systolic blood pressure

INTRODUCTION

Zinc is an essential mineral in the human body that is involved in several cellular processes. It is involved in nucleic acid synthesis, enzymatic reactions (being a component of more than 200 enzymes), cell replication and repair, and also plays an important role in energy producing functions.[1] Zinc has antioxidant and anti-inflammatory properties[2] and also known to play a key role in carbohydrate metabolism. Emerging evidence indicates that circulating levels of serum zinc may exhibit cardio-protective effects. In animal studies, zinc deficiency has been shown to elicit the release of pro-atherogenic factors[3] and supplementation with zinc has been shown to decrease the incidence of arrhythmias.[4] Observational studies have demonstrated associations between low serum levels of zinc and cardiovascular disease (CVD).[5-7] Zinc, in addition to its several physiological functions, has been reported to be involved in regulation of blood pressure.[8] Given the overall evidence, zinc may also be linked to hypertension ("high blood pressure"), which is the most common modifiable risk factor for CVD.[9] However, data on the association between serum zinc and hypertension are sparse, inconsistent, and have been based on only cross-sectional evidence. Some studies have demonstrated inverse associations between serum zinc and blood pressure,[7, 10, 11] others have shown positive associations,[12, 13] whereas others have shown no associations at all.[14] Due to the cross-sectional nature of the study designs of previous studies, the temporal sequence of the relationship between zinc and hypertension has not been established and it is not clear whether low serum zinc status increases the risk of hypertension among apparently healthy individuals. To date, no prospective evaluation of the association between serum concentrations of zinc and the development of hypertension has been published. Our primary objective was to evaluate in detail the nature and magnitude of the prospective association of serum zinc concentration with risk of incident hypertension in a population-based sample of 1,652 non-hypertensive men from eastern Finland. A secondary objective was to assess the association of dietary zinc intake with the risk of incident hypertension in the same set of participants.

METHODS

Study cohort

The study population consisting of a representative sample of men living in the city of Kuopio and its surrounding rural communities in Eastern Finland, were participants in the Kuopio Ischemic Heart Disease (KIHD) risk factor study; a longitudinal population-based study designed to investigate risk factors for cardio-metabolic outcomes and other chronic diseases.[15] Baseline examinations were performed between 1984 and 1989 and included men 42-61 years of age. Of 3433 potentially eligible and randomly selected men, 3235 were found to be eligible for the study. Of this number, 2682 (82.9 %) volunteered to participate, 186 did not respond to the invitation and 367 declined to give informed consent. Men with a prevalent history of hypertension were excluded. Prevalent hypertension was defined as having a clinical diagnosis of hypertension, systolic blood pressure (SBP) ≥ 140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg, or use of anti-hypertensive medication at baseline. The final cohort for the present analysis included 1,652 men with non-missing information on serum zinc and relevant covariates. The Research Ethics Committee of the University of Eastern Finland approved the study, and each participant gave written informed consent.

Risk factor assessment

Collection of blood specimens and the measurement of serum lipids, lipoproteins, glucose, and assessment of medical history and medications, smoking, and alcohol consumption have been described previously.[16] Blood samples were taken between 8 and 10 a.m. In addition to fasting, subjects were instructed to abstain from drinking alcohol for at least 3 days prior and from smoking for at least 12 hours. Fasting plasma glucose was measured by glucose dehydrogenase method (Merck, Darmstadt, Germany) after precipitation of proteins by trichloroacetic acid. Measurement of serum zinc concentrations were made from frozen serum samples stored at -20° C for 1-5 years, using the PerkinElmer 306 atomic absorption spectrophotometer (Norwalk, Connecticut, USA). Resting blood pressure was measured by an experienced nurse using a random-zero sphygmomanometer (Hawskley,

UK) after 5 and 10 minutes of rest in a seated position.[17] Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in meters. The energy expenditure of physical activity was assessed using the KIHD 12-month leisure-time physical activity questionnaire.[18, 19] Dietary zinc and energy intakes were assessed by recording food intake over 4 days using a questionnaire, and caloric intake of nutrients was calculated using Nutrica software (version 2.5; National Public Health Institute, Turku, Finland).[18] The Nutrica software mainly uses the Finnish values for the nutrients in the composition of food, taking into account the loss of vitamins during food preparation. It has a database on 1300 food items and dishes and 30 nutrients.

Ascertainment of incident hypertension

Incident hypertension was defined as a physician diagnosis of hypertension, SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg, or use of anti-hypertensive medication as determined at re-examination rounds 4, 11, and 20 years after the baseline and by record linkage to the national hospital discharge registry and to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for hypertension for the entire study period until the end of the follow-up.

Statistical methods

The analysis was pre-specified to exclude participants with a history of hypertension at baseline. We performed descriptive analyses summarizing baseline characteristics of participants. We assessed cross-sectional correlations of serum zinc concentrations with risk markers for hypertension using linear regression models adjusted for age. Time-to-event analyses were conducted using Cox proportional hazards models to examine the association of serum zinc with incident hypertension after confirming assumptions of proportionality of hazards.[20] The shape of the association of zinc with hypertension risk was assessed by plotting hazard ratios (HRs) calculated within quartiles of baseline serum zinc concentration against the mean serum zinc concentration within each quartile using floating absolute risks[21] as described previously.[22] Given the non-linear shape of the association, zinc was not

modelled continuously, but entered as fourths defined according to its baseline distribution. Because of the relatively flat risk of hypertension across quartiles 1-3 of serum zinc concentrations, these categories were combined and served as the reference comparison. Hazard ratios were adjusted for hypertension risk markers [age, BMI, SBP, smoking status, history of diabetes, total cholesterol, high-density lipoprotein cholesterol (HDL-C), alcohol consumption, physical activity, estimated glomerular filtration rate (eGFR), as calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula,[23] socioeconomic status, dietary zinc intake, and total energy intake]. We performed subgroup analyses using interaction tests to assess statistical evidence of any differences in HRs across levels of pre-specified individual level characteristics (such as age at survey, BMI, SBP, smoking status, history of diabetes, total cholesterol, HDL-C, eGFR, and dietary zinc intake). To avoid potential bias due to participants at high risk of or with underlying hypertension at baseline, we carried out additional analyses that excluded the first five years of follow-up. A11 statistical analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas).

RESULTS

Baseline characteristics

Baseline characteristics of the 1,652 participants without a known history of hypertension at baseline are shown in **Table 1**. The mean age of participants was 53.0 (standard deviation 5.1) years. Serum zinc concentrations were weakly and positively correlated with dietary zinc (r=0.04), physical measures (BMI, blood pressure, and physical activity), as well as several lipid and metabolic markers. Weak inverse correlations were observed for age (r = -0.13), HDL-C (r = -0.09), and eGFR (r=-0.05). Baseline serum zinc concentrations were lower by 3% in current smokers compared with non-current smokers.

Serum zinc concentrations and risk of incident hypertension

During a median follow-up of 24.7 years (35,894 person-years at risk), there were 259 incident hypertension cases (annual rate 7.22/1000 person-years at risk, 95% confidence interval (CI) 6.39 to 8.15). In analyses adjusted for age, a non-linear relationship was observed between serum zinc concentrations and incident hypertension risk. The shape of the association was similar on adjusting for several established risk factors (BMI, SBP, smoking status, history of diabetes, total cholesterol, HDL-C, alcohol consumption, and physical activity) (**Figure 1**). Comparing the top quartile versus bottom quartiles 1-3 of zinc concentration, the age-adjusted HR for hypertension was 1.65 (95% CI: 1.27 to 2.15; $P<0.001$), which was minimally attenuated to 1.48 (95% CI: 1.13 to 1.93; $P=0.004$) following further adjustment for hypertension risk factors. The results remained the same on additional adjustment for eGFR, socioeconomic status, dietary zinc intake, and total energy intake 1.47 (95% CI: 1.13 to 1.92; $P=0.005$) (**Table 2**). There were no new hypertension cases recorded during the first five years of follow-up, therefore results for analyses that excluded the first five years of follow-up were unchanged and therefore not shown. The associations generally did not vary significantly by levels or categories of several clinically relevant characteristics and other risk markers (P for interaction ≥ 0.10 for each; **Figure 2**).

To put our findings into context, comparisons were made to the association of dietary zinc intake with incident hypertension in the same set of participants. There was no evidence of an association of dietary zinc intake with risk of hypertension (**Table 3**).

DISCUSSION

Key findings

In this population-based cohort of middle-aged men with over 20 years of follow-up and without a history of hypertension at baseline, we have shown weak and generally positive associations of serum zinc concentrations with several risk markers for hypertension. Our data also suggest that higher baseline serum zinc concentration is positively and independently associated with incident hypertension in a non-linear fashion, though further work is required to determine whether a “U-shape” or “J-shape” would

better describe the relationship. These findings remained generally consistent across several clinically subgroups and at different levels of risk factors. We however found no evidence of an association of dietary zinc intake with incident hypertension.

Comparison with previous work

We were unable to locate any previously published articles exploring the prospective association between serum zinc and incident hypertension; therefore, it is not possible to compare our findings in the context of previous studies. Experimental and observational studies suggest that zinc is inversely associated with cardiovascular outcomes.[3-6] Evidence linking an association between zinc and blood pressure have mostly been based in animal models.[24-27] Few studies have been conducted in humans with inconsistent findings based on cross-sectional evidence.[7, 10, 14] However, given some of its physiological functions such as antioxidant and membrane-stabilizing effects,[28, 29] there are suggestions that zinc may be protective of high blood pressure. Therefore, it may appear our findings of a positive association are at odds with the biologically plausibility of the relationship. This is however not the case, as there is much controversy regarding the associations between zinc and several cardio-metabolic parameters and outcomes.[30] A number of experimental and observational studies have demonstrated associations between high serum zinc concentrations and adverse metabolic outcomes such as the metabolic syndrome[31-33] and diabetes.[34] Higher concentrations of serum zinc have also been associated with parameters such as obesity and lipids.[30, 32] Indeed, our study showed positive correlations between serum zinc and several cardio-metabolic indices. In addition, as demonstrated in our study, zinc has also been shown to have an inverse association with HDL-C.[30, 31] It is therefore possible that our findings may reflect a true association and also given the following strengths of our analyses: (i) the analysis was based on a large prospective population-based cohort study with an average follow-up of over 20 years with no loss to follow-up; (ii) participants were selected from a nationally representative sample of randomly selected men with a high response rate; (iii) men with a history of hypertension were excluded from the analyses; and (iv) there was information on a comprehensive panel

of lifestyle, biological markers, and dietary factors to allow adequate adjustment for potential confounding. However, given that this is the first prospective study to investigate this association, other large-scale prospective studies are still needed to confirm the current findings.

Possible explanations for findings

The link between zinc and blood pressure is still an under-researched area and the mechanistic pathways underlying this relationship are currently unclear. However, the literature is suggestive that zinc is involved in the regulation of blood pressure and in the development of hypertension.[8] In animal models, zinc is known to inhibit the ATP-dependent calcium pump which causes an outpour of calcium ions from the cell,[35] leading to a rise of free calcium ions in the smooth muscles of the vascular wall, subsequently leading to an increase in wall tension and hypertension. Zinc was also demonstrated to inhibit the activity of 1,4,5-triphosphoinositol-5-phosphatase ($InsP_3$),[36] causing accumulation of $InsP_3$, leading to an increase in intracellular calcium and subsequent increase in tension of arterial musculature.[26, 27] In addition, zinc deficiency has also been shown to lower the levels of angiotensin-converting enzymes[37] and reduce vasodilation reaction to bradykinin and prostacycline.[38] Finally, rats with an excess intake of zinc demonstrated impaired renal function with a concomitant increase in SBP, resulting from a decrease in the vasodilatory action of nitric oxide (which is known to be involved in the regulation of systemic blood pressure via vascular tone modulation[39]) triggered by superoxide radical-induced oxidative stress.[40] There is a possibility that these findings from animal models may be consistent with that observed in humans; however, further mechanistic studies are required to unravel the pathways involved in the association. Finally, there is a possibility that the association of serum zinc with incident hypertension may also reflect the different dietary patterns of the study population which include primary sources of zinc, which could potentially influence blood pressure. However, we adjusted for dietary factors such as total dietary energy intake as well as dietary zinc intake and we also found no evidence of an association between dietary zinc intake and hypertension in a subsidiary analysis. The null association demonstrated for dietary zinc intake and hypertension is not surprising, given that the

bioavailability of circulating zinc is much higher for zinc supplements than zinc from dietary sources.[41] Indeed, we observed a very weak correlation between serum zinc and dietary zinc intake in our study. In addition, dietary records which are assessed by self-reports are always prone to measurement error compared to circulating biomarkers assessed in serum. More studies are warranted to confirm these associations.

Strengths and limitations

In addition to the several strengths enumerated above, the KIHD study included men from an ethnically and genetically homogeneous population and employed reliable definitions of hypertension outcomes with information retrieved from established and reliable databases. The limitations also deserve mention. The study included middle-aged Caucasian men and hampers generalization of findings to younger men, women, and other populations. We accounted for several potential confounders including key clinical characteristics and dietary variables, but there is still a potential for residual confounding, as with all observational study designs. We only had a one-time measurement of serum zinc concentration, therefore, we could not correct for within-individual variation in serum zinc concentrations over time which may have underestimated the associations demonstrated. However, serum zinc has been demonstrated to display remarkable within-individual constancy.[42] Measurements of zinc concentrations in the KIHD study involved prolonged serum storage (1-5 years), which could have affected the stability of the samples. However, zinc concentrations have been shown not to be affected by prolonged storage in frozen serum samples (at -20°C) for several years or repeated freeze-thaw cycles.[43, 44]

Conclusions

This new prospective study demonstrates a positive and independent association between serum zinc concentration and incident hypertension in men. Our findings highlight a deleterious effect of higher serum zinc concentrations on blood pressure, contrary to suggestive notions that zinc may be protective

in the development of hypertension. Additional evidence is however needed to support these findings and assess the physiological mechanisms underlying the association.

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DISCLOSURES

None.

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Figure legends

Figure 1. Hazard ratios for incident hypertension by quartiles of baseline concentrations of serum zinc

A, adjusted for age; B, adjusted for age, body mass index, systolic blood pressure, smoking, history of diabetes, total cholesterol, high-density lipoprotein cholesterol, alcohol consumption, and physical activity; The size of the box is proportional to the inverse of the variance of hazard ratio.

Figure 2. Hazard ratios for incident hypertension comparing the top quartile versus bottom quartiles 1-3 of baseline concentration of serum zinc in subgroups of participants

*, *P*-value for interaction; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; Cut-offs used for age, body mass index, systolic blood pressure, total cholesterol, HDL-C, and dietary zinc intake represent median values.

Table 1. Baseline characteristics and cross-sectional correlates of serum zinc

	Mean (SD), median (IQR), or n (%)	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in zinc levels per 1 SD higher or compared to reference category of correlate‡
Serum zinc (mg/l)	0.93 (0.11)	-	-
Dietary zinc intake (mg/day)	14.9 (4.5)	0.04 (-0.01, 0.09)*	0% (-0, 1)
Questionnaire/Prevalent conditions			
Age at survey (years)	53.0 (5.1)	-0.13 (-0.18, -0.08)***	-2% (-2, -1)***
Alcohol consumption (g/week)	72.4 (138.9)	-0.16 (-0.22, -0.13)***	-2% (-2, -1)***
Total energy intake, kJ/day	2523 (663)	0.00 (-0.04, 0.05)	0% (-1, 1)
History of diabetes			
No	1,618 (97.9)	-	Ref
Yes	34 (2.1)	-	1% (-3, 5)
Smoking status			
Other	1,101 (66.7)	-	Ref
Current	551 (33.4)	-	-3% (-4, -2)***
Physical measurements			
BMI (kg/m^2)	26.3 (3.4)	0.16 (0.11, 0.20)***	2% (1, 2)***
SBP (mmHg)	129.8 (14.7)	0.10 (0.05, 0.14)**	1% (1, 2)***
DBP (mmHg)	85.8 (9.5)	0.08 (0.03, 0.13)**	1% (0, 2)*
Physical activity (kj/day)	1,532 (1,393)	0.06 (0.01, 0.11)	1% (0, 1)*
Lipid markers			
Total cholesterol (mmol/l)	5.92 (1.11)	0.06 (0.02, 0.11)*	1% (0, 1)*
HDL-C (mmol/l)	1.32 (0.31)	-0.09 (-0.13, -0.04)**	-1% (-2, -0)**
Triglycerides (mmol/l)	1.03 (0.76-1.46)	0.11 (0.07, 0.16)***	1% (1, 2)***
Metabolic and renal markers			
Fasting plasma glucose (mmol/l)	5.26 (1.06)	0.03 (-0.02, 0.08)	0% (-0, 1)
Serum creatinine (µmol/1)	88.8 (12.7)	0.05 (-0.00, 0.09)	1% (-0, 1)
Estimated GFR (ml/min/1.73 m^2)	87.7 (17.5)	-0.05 (-0.10, 0.00)	-1% (-1, 0)*

BMI, body mass index; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; SD, standard deviation; SBP, systolic blood pressure; †Pearson correlation coefficients between serum zinc and the row variables;. ‡Percentage change in zinc levels per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean zinc levels for the category versus the reference) adjusted for age; asterisks indicate the level of statistical significance: *, p<0.05; **, p<0.01; ***, p<0.001

Table 2. Associations of serum zinc concentrations and incident hypertension risk

Quartiles of zinc (mg/l)	**Events/ Total**	**Model 1**		**Model 2**		**Model 3**	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Q1-Q3 (0.50-1.00)	176 / 1,265	ref		ref		ref	
Q4 (1.01-1.62)	83 / 387	1.65 (1.27 to 2.15)	< 0.001	1.48 (1.13 to 1.93)	0.004	1.47 (1.13 to 1.92)	0.005

CI, confidence interval; HR, hazard ratio; ref, reference; Q, quartile

Model 1: Adjusted for age

Model 2: Model 1 plus body mass index, systolic blood pressure, smoking, history of diabetes, total cholesterol, high-density lipoprotein cholesterol, alcohol consumption, and physical activity

Model 3: Model 2 plus estimated glomerular filtration rate, socioeconomic status, dietary zinc intake, and total energy intake

Table 2. Association of dietary zinc intake and incident hypertension risk

Dietary zinc intake (mg/day)	**Events/ Total**	**Model 1**		**Model 2**		**Model 3**	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Per 1 SD increase	259 / 1652	1.06 (0.94 to 1.19)	0.360	1.06 (0.94 to 1.19)	0.362	1.04 (0.88 to 1.24)	0.654
Q1 (5.38-11.87)	63 / 415	ref		ref		ref	
Q2 (11.88-14.32)	49 / 411	0.79 (0.54 to 1.15)	0.221	0.81 (0.55 to 1.18)	0.267	0.83 (0.56 to 1.23)	0.352
Q3 (14.33-17.11)	75 / 414	1.15 (0.82 to 1.61)	0.425	1.15 (0.82 to 1.62)	0.419	1.19 (0.81 to 1.76)	0.380
Q4 (> 17.11)	72 / 412	1.22 (0.87 to 1.72)	0.255	1.25 (0.88 to 1.76)	0.214	1.34 (0.83 to 2.16)	0.237

CI, confidence interval; HR, hazard ratio; ref, reference; Q, quartile; SD, standard deviation

Model 1: Adjusted for age

Model 2: Model 1 plus body mass index, systolic blood pressure, smoking, history of diabetes, total cholesterol, high-density lipoprotein cholesterol, alcohol consumption, and physical activity

Model 3: Model 2 plus estimated glomerular filtration rate, socioeconomic status, dietary zinc intake, and total energy intake

Figure 1

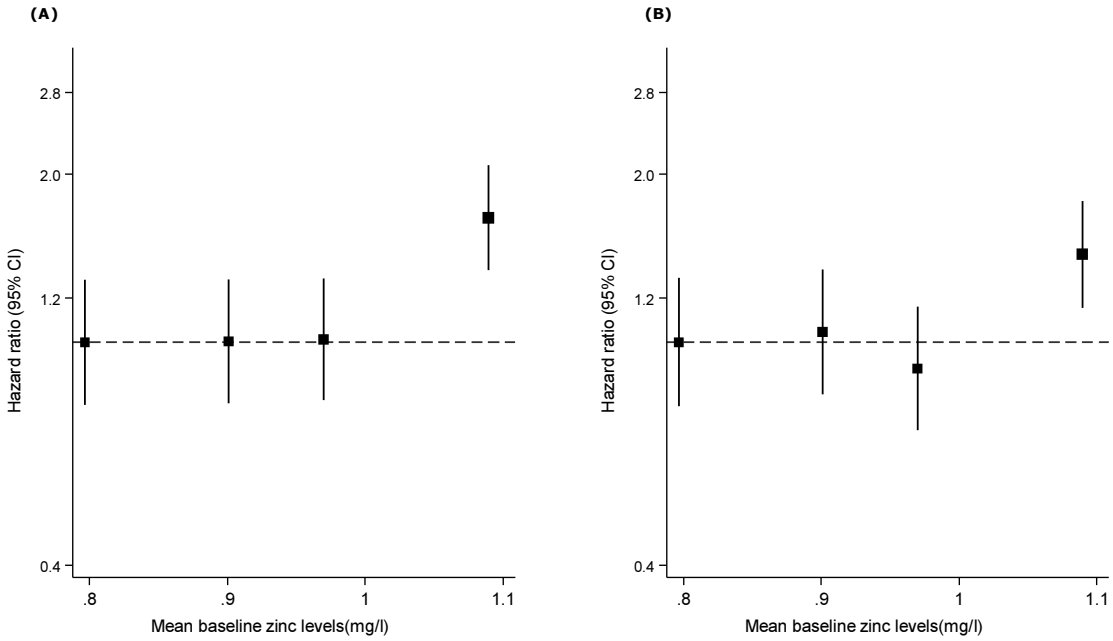
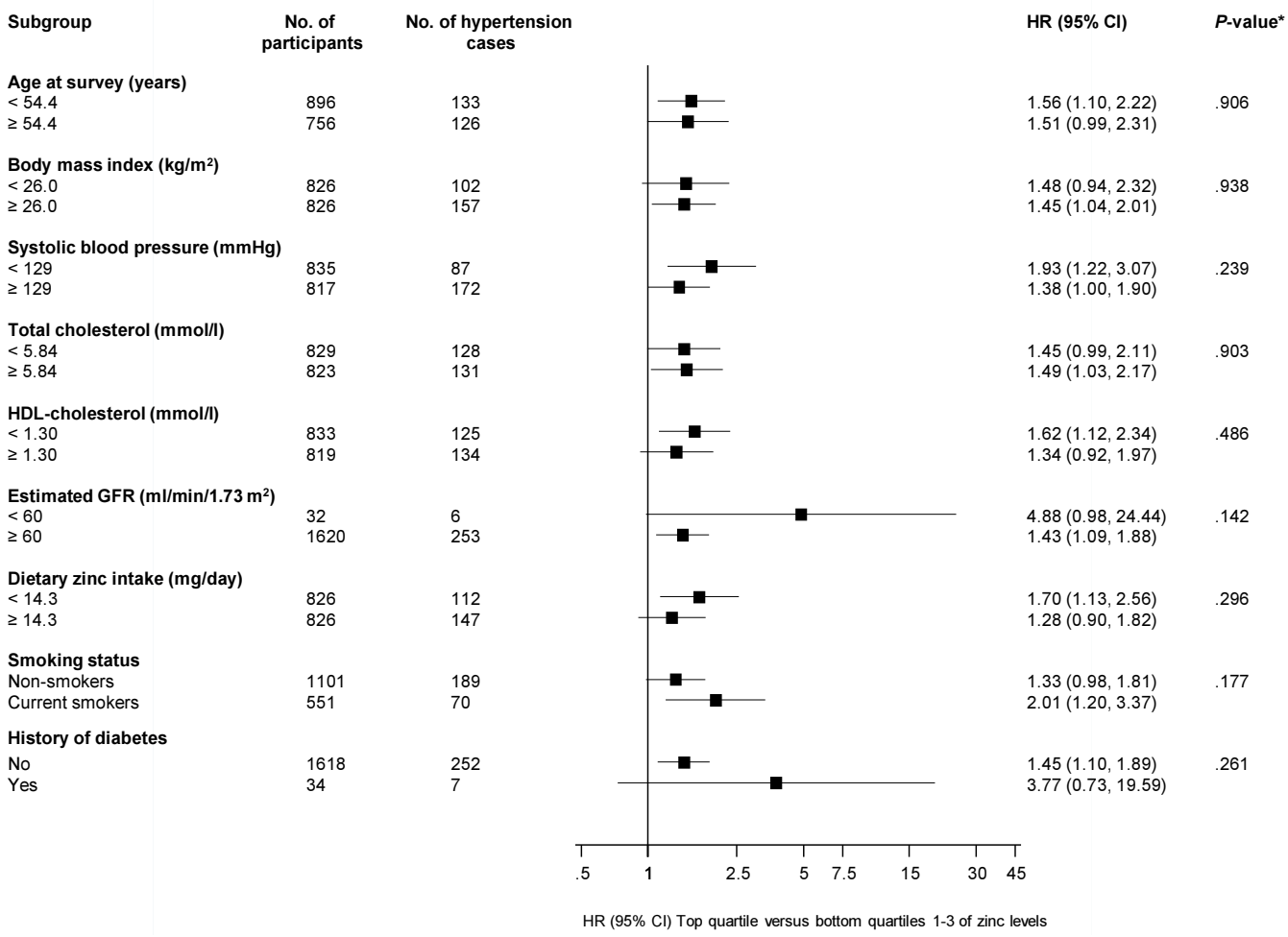

(A)
2.8
2.0
1.2
0.4
Hazard ratio (95% CI)
.8
.9
1
1.1
Mean baseline zinc levels(mg/l)
(B)
2.8
2.0
1.2
0.4
Hazard ratio (95% CI)
.8
.9
1
1.1
Mean baseline zinc levels(mg/l)

Figure 2


Subgroup
No. of participants
No. of hypertension cases
HR (95% CI)
P-value*
Age at survey (years)
< 54.4 896 133 1.56 (1.10, 2.22) .906
≥ 54.4 756 126 1.51 (0.99, 2.31)
Body mass index (kg/m²)
< 26.0 826 102 1.48 (0.94, 2.32) .938
≥ 26.0 826 157 1.45 (1.04, 2.01)
Systolic blood pressure (mmHg)
< 129 835 87 1.93 (1.22, 3.07) .239
≥ 129 817 172 1.38 (1.00, 1.90)
Total cholesterol (mmol/l)
< 5.84 829 128 1.45 (0.99, 2.11) .903
≥ 5.84 823 131 1.49 (1.03, 2.17)
HDL-cholesterol (mmol/l)
< 1.30 833 125 1.62 (1.12, 2.34) .486
≥ 1.30 819 134 1.34 (0.92, 1.97)
Estimated GFR (ml/min/1.73 m²)
< 60 32 6 4.88 (0.98, 24.44) .142
≥ 60 1620 253 1.43 (1.09, 1.88)
Dietary zinc intake (mg/day)
< 14.3 826 112 1.70 (1.13, 2.56) .296
≥ 14.3 826 147 1.28 (0.90, 1.82)
Smoking status
Non-smokers 1101 189 1.33 (0.98, 1.81) .177
Current smokers 551 70 2.01 (1.20, 3.37)
History of diabetes
No 1618 252 1.45 (1.10, 1.89) .261
Yes 34 7 3.77 (0.73, 19.59)
.5
1
2.5
5
7.5
15
30
45
HR (95% CI) Top quartile versus bottom quartiles 1-3 of zinc levels